Calcium Dobesilate Inhibits the Alterations in Tight Junction Proteins and Leukocyte Adhesion to Retinal Endothelial Cells Induced by Diabetes

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OBJECTIVE—Calcium dobesilate (CaD) has been used in the treatment of diabetic retinopathy in the last decades, but its mechanisms of action are not elucidated. CaD is able to correct the excessive vascular permeability in the retina of diabetic patients and in experimental diabetes. We investigated the molecular and cellular mechanisms underlying the protective effects of CaD against the increase in blood–retinal barrier (BRB) permeability induced by diabetes.

RESEARCH DESIGN AND METHODS—Wistar rats were divided into three groups: controls, streptozotocin-induced diabetic rats, and diabetic rats treated with CaD. The BRB breakdown was evaluated using Evans blue. The content or distribution of tight junction proteins (occludin, claudin-5, and zonula occluden-1 [ZO-1]), intercellular adhesion molecule-1 (ICAM-1), and p38 mitogen-activated protein kinase (p38 MAPK) was evaluated by Western blotting and immunohistochemistry. Leukocyte adhesion was evaluated in retinal vessels and in vitro. Oxidative stress was evaluated by the detection of oxidized carbonyls and tyrosine nitration. NF-κB activation was measured by enzyme-linked immunosorbent assay.

RESULTS—Diabetes increased the BRB permeability and retinal thickness. Diabetes also decreased occludin and claudin-5 levels and altered the distribution of ZO-1 and occludin in retinal vessels. These changes were inhibited by CaD treatment. CaD also inhibited the increase in leukocyte adhesion to retinal vessels or endothelial cells and in ICAM-1 levels, induced by diabetes or elevated glucose. Moreover, CaD decreased oxidative stress and p38 MAPK and NF-κB activation caused by diabetes.

CONCLUSIONS—CaD prevents the BRB breakdown induced by diabetes, by restoring tight junction protein levels and organization and decreasing leukocyte adhesion to retinal vessels. The protective effects of CaD are likely to involve the inhibition of p38 MAPK and NF-κB activation, possibly through the inhibition of oxidative/nitrosative stress. ***Diabetes*** **59:2637–2645, 2010**

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The blood–retinal barrier (BRB) breakdown is the hallmark of diabetic retinopathy (1). Alterations in BRB occur early in the progression of diabetic retinopathy and eventually lead to macular edema, which is responsible for vision loss (2). The increase in BRB permeability is associated with changes in the expression, content, phosphorylation, and distribution of tight junction proteins in retinal vessels (3–7), as well as with increased vesicular transport mediated by endocytotic vesicles (8).

Occludin and claudins are responsible for the direct cell-to-cell attachment in the tight junction barrier (9,10) and are a crucial determinant of tight junction permeability properties in endothelial cells (11,12). Claudin-5 is necessary to preserve the vascular barrier to small (<0.8 kDa) molecules in the brain (13), and it possibly also plays a similar role in the BRB. The zonula occluden proteins (ZO-1, -2, and -3) coordinate the assembly of the junctional complex and provide the interaction with components of the cytoskeleton (14), also important for BRB function.

Diabetes causes metabolic and physiologic abnormalities in the retina, and it appears that inflammation plays a critical role in the development of diabetic retinopathy. Those changes include the upregulation of inducible nitric oxide synthase, *cyclooxygenase*-2, intercellular adhesion molecule-1 (ICAM-1), caspase-1, vascular endothelial growth factor (VEGF), and nuclear factor kappa B (NF-κB), as well as increased production of nitric oxide, prostaglandin E2, and cytokines (15,16). We and others also demonstrated that the adhesion of leukocytes to retinal vessels is increased in the retinas of diabetic animals, and this increase is correlated with changes in tight junction proteins and increased BRB permeability (4,6,8,17,18). The increase in leukostasis is also associated with an increase in the expression of ICAM-1 by retinal endothelial cells (18,19). NF-κB regulates the expression of adhesion molecules, such as ICAM-1, and NF-κB activation has been correlated with the increase in leukostasis and BRB breakdown in diabetic rat retinas (20). Moreover, the p38 mitogen-activated protein kinase (p38 MAPK), a stress-activated serine/threonine protein kinase, is activated in response to proinflammatory cytokines and oxidative stress. The activation of p38 MAPK has been reported in the retinas of diabetic rats and is associated with BRB breakdown (21).

Calcium dobesilate (CaD) is considered an angioprotective drug, and it has been used in the treatment of diabetic retinopathy and chronic venous insufficiency in several countries during the last few decades (22,23), but its efficacy in the treatment of diabetic retinopathy is still a